GROWTH AND REPAIR OF SPINES IN THE SEA URCHIN STRONGYLOCENTROTUS PURPURATUS (STIMPSON)[1]

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The following work represents a number of observations concerning the growth and repair of spines on the West Coast purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson). Some of the parts were introductory sorties into a study of natural urchin populations; others were incidental to the main study of urchin ecology and simply suggested themselves as the research progressed.

An examination of the literature concerning the growth of urchin spines suggests that two issues have attracted workers: the presence of growth lines or cycles in the spines which has led to the hope of using them to determine the age of animals; and, secondly, the phenomenon of regeneration of entire spines or spine tips.

Spine sections have been described for many species of echinoids by various authors (Carpenter, 1847, 1870; Mackintosh, 1879, 1883a, 1883b; Kříženecký, 1917; Deutler, 1926; Mortensen, 1928–1951; Swan, 1952; McRae, 1959; Moore, 1966) and the rings, cycles of wedges, or growth lines which appear in cross section have had various interpretations. Carpenter (1847, 1870) and Swan (1952) suggested that these cycles may be formed like the annual growth layers in woody perennial plants. Moore (1966) found a positive correlation between numbers of rings and test volume, and suggested that the rings might be used to determine age in *Heliocidaris erythrogramma*. Deutler (1926) called these rings "growth zones" and thought that they were formed periodically in *Echinus*.

Regeneration of broken spines was apparently first suggested by Quekett (cited by Carpenter, 1870). The first sections of spines showing repair and interpreted as such were made by Carpenter (1870) with *Echinus trigonarius* and *Acrocladia* sp. Borig (1933), using *Echinometra mathaei*, recognized that cycles shown in longitudinal section ended in sharp discontinuities which were the result of breaks and subsequent regeneration. He concluded that cycles were not directly related to regeneration but rather that the spine would not regenerate a new tip until the next "growth period" and that, at this time, cycles would be added even though no break had occurred. The only other reference connecting regeneration with the number of cycles is Swan (1952), who found completely regenerated spines did not have the same number of cycles as original ones. Swan also pointed out that some externally purple spines of *S. purpuratus* had green cores and that regenerated spines were purple, unlike the spines of young animals which were green. The implication was that it should be possible to determine whether a spine was one that had completely regenerated or had grown from the original small spine

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of a young urchin. Part of the puzzle in examining such spines is the sharp distinction between green and purple, which are separated by a cycle of calcite crystals.

MATERIALS AND METHODS

The West Coast purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson) was used in the following studies and all collections were made at the south side of Sunset Bay, Coos County, Oregon, 43° 20′ N. lat. Because various techniques were employed in the different portions of this work, for the sake of continuity, methods will be discussed where appropriate in each section of the results.

RESULTS

A. *Relationship between internal spine morphology and animal size*

At the beginning of this investigation in 1962, it was hoped that cycles present in spines could be used to determine the age of animals. As a first step, the relationship between urchin diameter and number of rings in the spines was examined.

TABLE I

Number of cycles in green-cored spines on urchins collected from Sunset Bay December 8, 1962

Animal number	1	2	3	4	5	6	7	8	9	10	11	12
Test diameter	7.71	6.20	6.20	4.69	4.57	3.51	2.65	2.41	2.41	2.20	2.11	2.09
Number of cycles	9	8	8	6	8	6	6	5	5	5	5	5

Correlation coefficient $r = 0.91$.

In December, 1962, four spines were taken in the field from each of 33 animals. Spines were dried in an oven overnight, dipped in xylene and mounted on microscope slides with "Permount." After hardening, the preparations were ground in longitudinal section. Cycles or rings were counted only in green-cored spines and, when such a spine was found for any animal, no further sections were prepared.

Of the 33 urchins examined, only 12 showed at least one green-cored spine. A positive relationship existed between test diameter and number of cycles (Table I) with a correlation coefficient of 0.91. It was this initial relationship which seemed to promise that age of individuals in years could be obtained from spine morphology, and stimulated further work towards finding the time of year when a cycle would be formed.

B. *A general examination of spine morphology*

Initial efforts to determine the season when new rings were formed was frustrated by lack of a suitable tagging method that would permit individuals in the field to be followed for at least one year. After this problem was solved in the summer of 1963 (Ebert, 1965), 100 animals were marked, a sample of five large primary spines removed from the ambitus of each tagged animal, and the urchins placed in a pool at Sunset Bay. The original plan was to examine the spines and sample more from the same animals at several periods during the year to discover when new cycles were formed.

The handling of spines for preparation of longitudinal sections was modified and eventually approached the techniques of Carpenter (1847) and Deutler (1926) with certain differences. Most of the organic material was removed from the spines with a solution of 5.25% NaOCl (full strength household bleach) as suggested by Swan (1952). If organic material was not removed from the spines, they became brittle when dry and usually fractured during the grinding process. After washing in water for at least three hours and drying, the spines were dipped in xylene, placed on a slide and covered with Canada balsam. They were heated on an electric hot-plate to boil away the xylene, and cooled. When hard, the preparations were ground on a glass plate with #220 followed by #600 carborundum grinding compound. Water was used as the liquid carrier. The slide was tilted during the grinding process to insure the production of a median section. After grinding one side, the slide was returned to the hot-plate, the balsam remelted and the spine turned over, recooled and the grinding completed. The preparation was cleaned in xylene before the coverslip was added. In this manner, 500 sections were prepared.

Figure 1 shows typical sections of these 500 spines. Figure 1A is a cross section showing 10 cycles or rings and is a typical presentation of a so-called polycyclic spine. Each light block (in the outer cycle there are 45) is a single calcite crystal which runs generally parallel with the long axis of the spine. The outside ring of these crystals gives the spine a ribbed or fluted appearance. Between the large crystals are smaller ones which form a meshwork. The dark color is due to the presence of echinochrome.

Figure 1B is a cross section of a spine from the same animal as 1A and shows 4 cycles (52 blocks in the outer cycle). The inner region of the spine is constructed of a loose calcite meshwork. Figures 1C–E are longitudinal sections, are from different animals, and show variations in the arrangements of the cycles of large crystals.

Figure 1C is a portion of a spine tip with the bottom of the picture being towards the base of the spine. The light lines are the large crystals which form the cycles, and the dark regions are fine meshworks of calcite with echinochrome pigment. Distally, the large crystals end abruptly; several fuse towards the base which means, in cross section, that two cycles become one. A major discontinuity is shown towards the bottom of the picture.

Figure 1D again shows large crystals ending abruptly at their distal ends and, towards the tip of the spine, partial fusion of a number of cycles. At the base of the spine, the 3rd, 4th and 5th crystals (starting the count from the outside) all fuse distally to form a single crystal a short distance above the milled ring. The milled ring, a region of muscle attachment, is indicated by an arrow. The fusion of crystals near the base apparently was the nature of the entire cycle because the pattern appears on both sides of the spine. One further important feature is the shape of the large crystals. About in the center of 1D are several bent crystals whose shape must have changed during growth.

In Figure 1E there is a major discontinuity immediately above the milled ring (arrow) which terminates 7 cycles. The outer cycle encloses a fine meshwork of

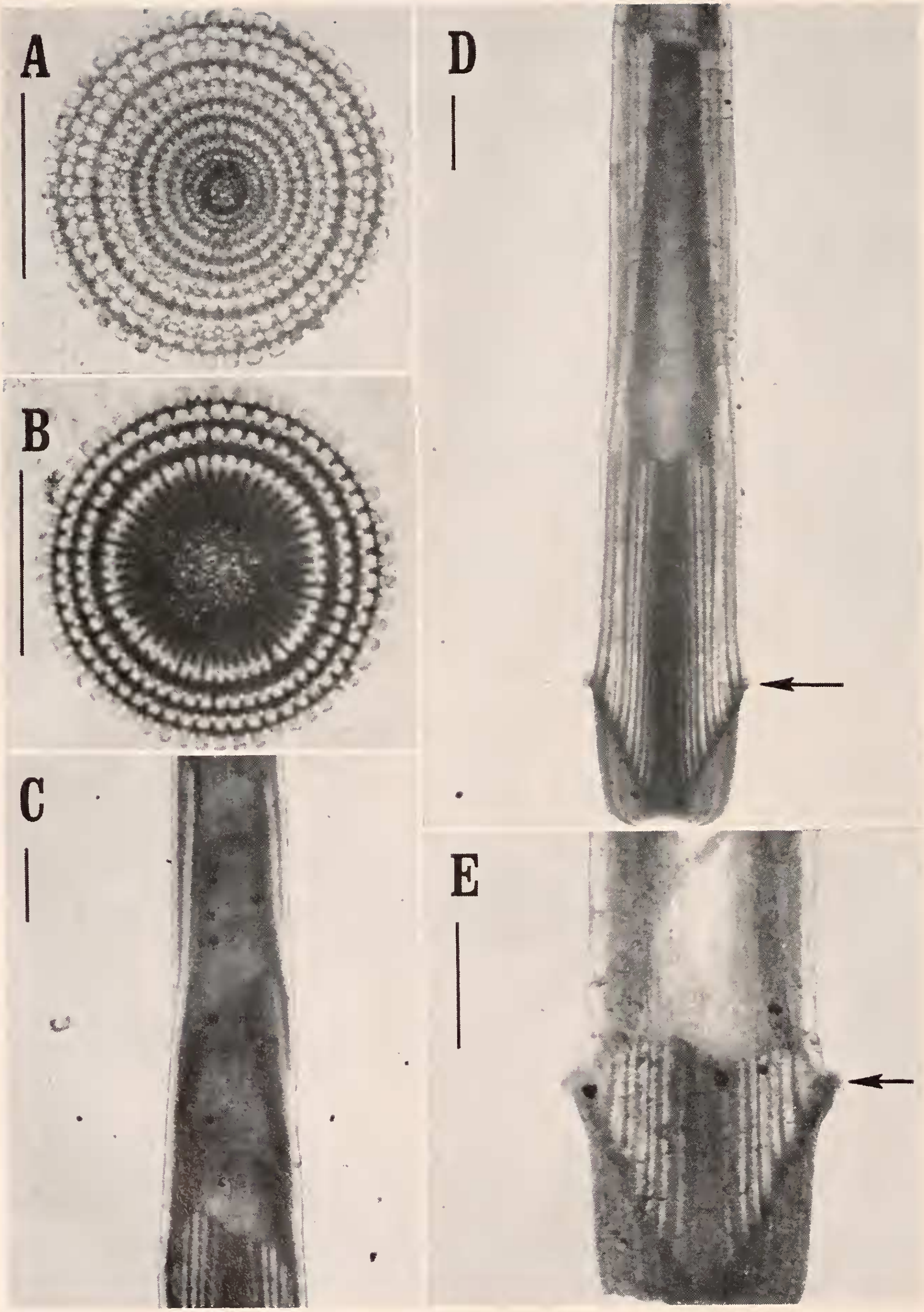

Figure 1.

calcite; however, the central region is coarser in texture and apparently structurally weaker. Spines of this type usually lost this central region during grinding.

Spines have been examined at various times during the year and the morphology of the spines was never different than described above.

C. *Formation of discontinuities between green and purple in spines*

On October 2, 1963, urchins of age class 0, *ca.* 1 cm. in test diameter, were observed at Sunset Bay. One group of 40 animals in a small tidepool was completely covered with debris consisting of shells, shell fragments and pieces of algae. All of these animals were green. A second group, also of age class 0 and consisting of several hundred individuals, was living in a large tidepool and not covered with debris. All of these were purple. A possible explanation was that the production of echinochrome was, in some fashion, related to light. In early marking experiments, there was an indication that pigment production was also related to injury or that echinochrome was moved to areas of irritation. A simple experiment was designed to investigate both light and injury. Two 1-gallon glass jars were filled $\frac{3}{4}$ full of sea water and placed in an 11° C. constant temperature room. Eight age class 0 urchins were prepared for each jar: four were green and the other half purple. Spine tips on two green and two purple animals of each group were clipped. The animals were photographed and placed in the jars which were then sealed. A small hole was allowed in each lid to permit the passage of a rubber hose for an air supply. Aluminum foil was wrapped around one jar, occluding all light, and a 100-watt lamp was placed one foot away from the other. The experiment was continued for one month. Animals were again photographed on November 21 and December 2, at which times the urchins in the darkened container were, of course, briefly exposed to light.

Results of spine growth after one month under conditions of darkness showed regenerating spine tips were white to greenish on both green and purple urchins. Under light conditions, regenerating spine tips were purple in both green and purple urchins. The first conclusion is that light is important in echinochrome synthesis or transport and, secondly, that a sharp discontinuity can arise between green and purple in young spines through breakage and regeneration under light conditions. This would mean that the sharp line at the top of a green core represents a break and regeneration of a small urchin's spine under light conditions.

D. *Relationship between regeneration and internal spine morphology*

A further examination of the role of regeneration in determining the distribution of cycles in spines was started on May 4, 1964, when a sample of urchins was collected and brought back to the University of Oregon, placed in aquaria of aerated sea water and kept at 11° C. Four animals (4.15–7.15 cm. test diameter) were individually marked with nylon monofilament through an ambulacrum (Ebert, 1965) and returned to the aquaria. Tips of all primary spines in the interambulacrum next to the mark were removed and placed on file cards in the order of

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FIGURE 1. Ground sections of *S. purpuratus* spines. A and B are cross sections taken above the milled ring; C–E are longitudinal sections; the milled ring is indicated by an arrow. The scale lines are 1 mm. Black spots are particles of grinding compound.

removal on May 8. The position of the mark on each animal was mapped to insure proper matching of the tips with the spines at a later date. Spines were collected again on July 8, 1964, cleaned with NaOCl, matched with their previous tips, mounted on slides and ground in longitudinal section so that the cycles could be examined.

Figure 2 shows a typical pairing of a spine tip with its original spine after two months of regeneration. A new cycle of calcite crystals formed during this time. The break shows as a discontinuity in the regenerated spine similar to the sharp lines seen in the spines in Figure 1.

Discussion

Discussions by Mortensen (1928–1951) and Swan (1952) during the initial stages of this work, to a great extent, were responsible for the original hope of being able to determine age of animals by examining spine morphology. A further consideration which strengthened this belief was the positive correlation between size of animal and number of cycles in green-cored spines. After examining a large number of spines during 1963, I was led to form an alternate hypothesis for the formation of cycles, *viz.* that they were formed when a spine tip was broken and regenerated. This was proposed because: (a) spines always had a cycle of crystals on the outside. If the cycles were formed only at certain periods during the year, then, at some time, the fine crystalline meshwork would have to appear on the outside; (b) in longitudinal section, the cycles were always distally terminated at a sharp discontinuity which suggested a break. The two experiments with regeneration in the laboratory, production of the sharp color discontinuity in spines of small animals and the addition of a new cycle of calcite crystals in larger animals, conclusively showed that the alternate hypothesis was correct and that cycles have no direct relationship with age other than the very simple one, that breaks tend to accumulate in a spine through time. Old animals, as a result, would tend to have more cycles in their spines than would young animals. This is the proper interpretation of the positive correlation between number of cycles and size. Spines that have been completely regenerated would show fewer cycles and no green core, as suggested by Swan (1952).

Although no work has been done on the histology of the spines during growth and regeneration, some ideas can be presented concerning possible occurrences which would be consistent with observed spine sections. Following a break, the dermis on the remaining spine stump must be sloughed off or altered in some fashion so that when regeneration begins, the former outer ring of large crystals is simply covered over by new growth. A new dermis probably is formed of cells originating from within the existing spine shaft rather than growing up from the milled ring, because regeneration appears to begin on the broken stump. That the dermis is injured in some fashion seems consistent with the partial regeneration lines seen in Figure 1C and D. Partial regeneration would result if damage was not great enough to affect the dermis of the entire spine. At the top of Figure 1D, a series of four regeneration lines merge into a single line. The arrangement is best interpreted as multiple regeneration where a small break occurred and was repaired (the innermost cycle at the top of the spine). Growth of a new tip took place with new outer crystals merging with original outer crystals. The tip

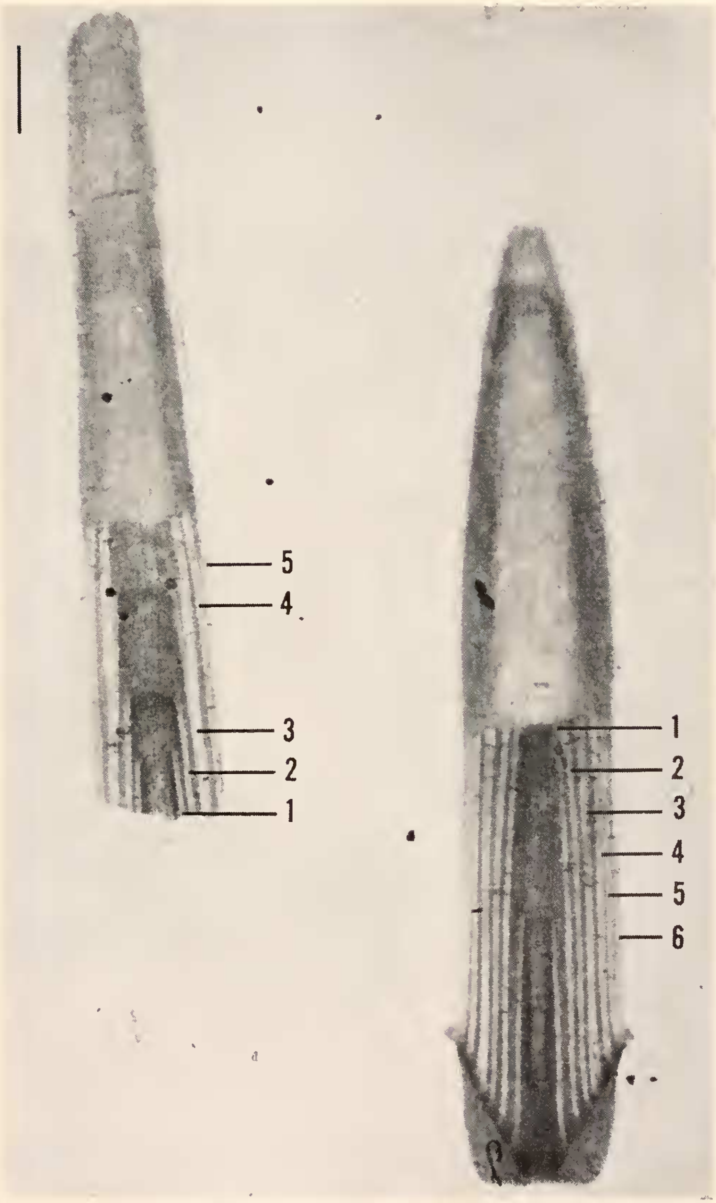

FIGURE 2. Matched tip and regenerated spine. The cycles are numbered and indicate that during regeneration of a new tip, a new cycle was added (6). The scale line is 1 mm. Black spots are particles of grinding compound.

was broken again at the same point as the first break. The extent of influence of the break apparently was greater, and the next set of calcite crystals was added further down the shaft of the spine. This sequence was repeated until the effect of breakage was enough to cause a cycle to be added all the way to the milled ring. That a spine would break at the same place a number of times is not too unlikely because new material formed at the site of a break is apparently weaker than that deposited later. Mackintosh (1879) suggested that the hollow appearance of certain spines was associated with repair. Carpenter (1847, 1870) also felt that there was a relationship between large regions of regeneration and formation of structurally weak calcite meshworks. Such weakened zones, as shown in Figure 1E, would be likely sites for multiple breaks.

Kříženecký (1916) suggested that when a spine was broken near the tubercle, it was shed and an entire new spine was formed. This seemed to be generally true in this study; however, as Figure 1E indicates, a break can occur almost to the milled ring and the spine still regenerate. It is possible that the critical factor for determining spine loss after breakage may not be the absolute amount of material lost but rather the damage done to underlying tissues such as the inner and outer muscle layers attached to the base of the spine. The fusion of calcite crystals in Figure 1D near the milled ring may have resulted from damage to the supporting tissues of the spine. In such a case, damage would not have been great enough to cause spine loss.

It now seems possible that ideas concerning the growth of echinoid spines must be modified. Spines may not grow simply by addition of new material to the outside. Large calcite crystals forming a cycle are always on the outside of the spine, yet the spine increases in size and a fine mesh of calcite develops between the outside cycle of large crystals and the next inner cycle. Furthermore, calcite crystals, if bent, may be straightened during growth, as suggested by Figure 1D. All calcite parts are bound together in a three-dimensional lattice, and so, to maintain the observed arrangement, calcite must be resorbed as well as deposited. Resorption of test plates has been discussed by Lovén (1892), Jackson (1912), Deutler (1926), Gordon (1926), and Mortensen (1927), and was experimentally shown by Cutress (1965). The highly precise and dynamic nature of growth, however, was not indicated by previous workers. Growth of spines, as shown in this study, may be as complex as vertebrate bone growth with its interaction of osteoblasts and osteoclasts. Ordered resorption and deposition in other invertebrates have been indicated in brachiopods (Hyman, 1959; p. 529) and, to an apparently lesser extent, in molluscs (Wilbur, 1964).

SUMMARY

1. Numbers of cycles in the spines of *S. purpuratus* were positively correlated with animal size.

2. Examination of 500 spine sections in this study led to the hypothesis that cycles are the result of breakage and regeneration.

3. The relationship between breakage, regeneration and internal spine morphology was established by producing sharp lines of color in the spines of young urchins and new cycles in the spines of larger animals, both as a result of regeneration.

4. From observed morphology and experimental evidence, it appears that growth of spines requires a very precise resorption as well as deposition of calcite.

LITERATURE CITED

Borig, P., 1933. Über Wachstum und Regeneration der Stacheln einiger Seeigel. *Z. Morphol. Ökol. Tiere,* **27**: 624–653.

Carpenter, W. B., 1847. Report on the microscopic structure of shells. British Assoc. Adv. Sci., London. 1847: 115 + Figure 65 dd, ee.

Carpenter, W. B., 1870. On the reparation of the spines of echinida. *Monthly Microsc. J.,* **3**: 224–228 + Plate XLIX.

Cutress, B. M., 1965. Observations on growth in *Eucidaris tribuloides* (Lamarck), with special reference to the origin of the oral primary spines. *Bull. Mar. Sci.,* **15**: 797–834.

Deutler, F., 1926. Über das Wachstum des Seeigelskeletts. *Zool. Jahrb.* (*Anat. u. Ont.*), **48**: 119–200.

Ebert, T. A., 1965. A technique for the individual marking of sea urchins. *Ecology,* **46**: 193–194.

Gordon, I., 1926. The development of the calcareous test of *Echinus miliaris. Phil. Trans. Roy. Soc., London, Series B,* **214**: 259–312.

Hyman, L. H., 1959. The Invertebrates. V. Smaller Coelomate Groups. McGraw-Hill Book Co., Inc., New York.

Jackson, R. T., 1912. Phylogeny of the echini with a revision of Palaeozoic species. *Mem. Boston Soc. Nat. Hist.,* **7**: 1–490.

Kříženecký, J., 1916. Ein Beitrag zur Kenntnis der Regenerationsfähigkeit der Seeigelstacheln. *Arch. f. Entw.,* **42**: 642–650 + Plate 38.

Lovén, S., 1892. Echinologica. *K. Svenska Vetensk Akad. Handl., n.s.* **18**: 1–74 + Plates 1–12.

Mackintosh, H. W., 1879. The acanthology of the Desmosticha Pt. I. *Trans. Roy. Irish Acad.,* **26**: 475–490.

Mackintosh, H. W., 1883a. The acanthology of the Desmosticha Pt. II. *Trans. Roy. Irish Acad.,* **28**: 241–258 + Plates 5–8.

Mackintosh, H. W., 1883b. The acanthology of the Desmosticha Pt. III. *Trans. Roy. Irish Acad.,* **28**: 259–266 + Plates 9–10.

McRae, A., 1959. *Evechinus chloroticus* (Val.), an endemic New Zealand echinoid. *Trans. Roy. Soc. N. Z.,* **86**: 205–267.

Moore, G. P., 1966. The use of trabecular bands as growth indicators in spines of the sea urchin, *Heliocidaris erythrogramma. Aust. J. Sci.,* **29**: 52–54.

Mortensen, T., 1927. On the postlarval development of some cidarids. *D. K. Danske Vidensk. Selsk. Skr., Naturvidensk. Mathem. Afd., ser. 8,* **11**: 369–387.

Mortensen, T., 1928–1951. A Monograph of the Echinoidea. I–V + index. Copenhagen: C. A. Reitzel; London: H. Milford, Oxford Univ. Press.

Swan, E. F., 1952. Regeneration of spines by sea urchins of the genus *Strongylocentrotus. Growth,* **16**: 27–35.

Wilbur, K. M., 1964. Shell formation and regeneration. *In:* Physiology of Mollusca Vol. I, K. M. Wilbur and C. M. Yonge eds., Academic Press, New York. xiii + 473.